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SEX DIFFERENCES IN THE PHENOTYPIC CHARACTERISTICS OF RAT THYMOCYTES

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This study was undertaken to assess whether there is any difference in the process of intrathymic T cell differentiation that could account for sexual dimorphism in the immune response. To accomplish this aim the relative proportion of four main thymocyte subsets delineated by expression of CD4 and CD8 surface markers was studied in rats of both sexes, using appropriate monoclonal antibodies and direct two-color flow cytometric analysis (FCA). Although the total yield of T cells extracted from thymuses did not differ between the sexes, in females was found a decreased percentage of double positive (DP) $CD4^+/CD8^+$ cells accompanied by a proportional increase in the percentage of single positive (SP) $CD4^+/CD8^-$ cells. The results indicate that the balance of intrathymic T cell maturation in females is shifted in faor of the helper / inducer subset. Moreover this shift in balance might be, at least partly, responsible for the phenomenon termed immunological sexual dimorphism.

Key words: sexual dimorphism, T cell differentiation, thymus

INTRODUCTION

Clinical and experimental evidence in both humans and animals suggest that gonadal steroids are capable of modulating the immune response (Grossman, 1985; Grossman, 1990). Also a bulk of evidence substantiates the presence of naturally occurring sexual dimorphism in many immunological parameters (Grossman et al., 1985). Females seem to have a more vigorous immune response (Homo-Delarche and Dardenne, 1993). Thus, the concentration of immunoglobulins is higher in females, the primary and secondary immune response is stronger, they are more resistant to the induction of immunological tolerance and they show greater ability to reject tumors and allografts (Homo-Delarche and Dardenne, 1993). It has been assumed that this sex-related difference in the immune response might be due to the different ratio of sex hormones in females and males rather than to sex chromosome-linked genes (Dumont, 1985). However, data which would elucidate the hormonal mechanisms accounting for the immune dimorphism still are very limited.

Having in mind that the thymus orchestrates the functioning of the immune system controlling T cell maturation, as well as that the thymic cells bind sex steroid hormones (Reichman and Villel, 1978; Grossman et al., 1979; Kawashima et al., 1991; Pearce et al., 1981; Kovacs and Olsen, 1987), it can be expected that different sex hormones present within the thymic microenvironment may have distinct effects on T cell maturation, hence inducing sexual dimorphism in the immune response. To assess this hypothesis the relative proportion of the four main thymocyte subsets delineated by CD4 and CD8 surface markers has been estimated in both male and female rats of the same age.

MATERIALS AND METHODS

Animals: Adult, 75 day old, male and female rats of the inbred AO strain housed in the vivarium of the Military Medical Academy, Belgrade, YU were used in the present experiment. The animals were sacrificed by decapitation and their thymuses were removed and dissected free of parathymic lymph nodes and adherent membranous tissue with extreme care.

Preparation of thymic cell suspensions

The thymic lobes were excised and placed in individual Petri dishes containing ice cold phosphate-buffered saline (PBS). The thymocyte suspension was prepared by grinding the thymic tissue between the frosted ends of microscope slides and passing the resultant suspension through a fine nylon mesh. The single-cell suspension obtained thus was washed three times in ice-cold PBS (pH 7.3) containing 2% fetal calf serum (GIBCO Laboratories, Grand Island, NY) and 0.01% sodium azide (PS medium). Then the cells were enumerated in a standard hemocytometer to determine the thymic cellularity and resuspended in an appropriate volume of PS medium. The viability of such cell preparations, as determined by trypan blue dye exclusion was routinely greater than 95%. The purity of the cell population was checked using a mAb (clone MRC OX-19, Serotec Ltd, Oxford, England) that recognizes a determinant expressed on all thymocytes and peripheral T cells, but does not bind to B cells, macrophages, NK cells, mast cells or other cell types by direct one-color FCA. The relative number of cells binding this mAb routinely exceeded 95%.

Flow cytometry: Aliquots of 1x 10^6cells in 100 μl of PS medium were dispensed in conical microcentrifuge tubes, centrifuged to form a pellet and the supernatant decanted. The cells were resuspended and then incubated 30 min on ice in 20 μl of PS medium supplemented with fluorescein isothiocyanate (FITC) - conjugated anti CD4 (clone W3/25, Serotec Ltd, Oxford, England) and phycoerythrin (Pe) - conjugated anti CD8 (clone MRC OX8, Serotec Ltd, Oxford, England) monoclonal antibodies (mAbs). The mAbs were previously titrated to optimal concentrations at which no cellular aggregation was detected. After the incubation with mAbs the cells were washed three times in ice-cold PS medium, fixed in 0.5 ml of 1% paraformaldehyde and kept at 4^0 C in the dark until analysis. All samples were analyzed on the same day on a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA). Dead cells and debris were excluded from analyses by selective gating based on anterior and right angle scatter. 10^4 flow cytometer events were analyzed with respect to appropriate isotypic and fluorochrome-matched controls, using Consort 30 and Lysis software. All data

were collected and displayed in a log scale of increased green and red fluorescence intensity. Data were presented as two-dimensional contour maps. To obtain the percentage of thymocyte subpopulations, total counts were integrated in selected areas of the contour plots.

Statistical analysis: The relative proportion of each thymocyte population was compared between males and females by Student's t-test. A p value of less than or equal to 0.05 was considered significant. The data were plotted using the scientific Harward graphics program.

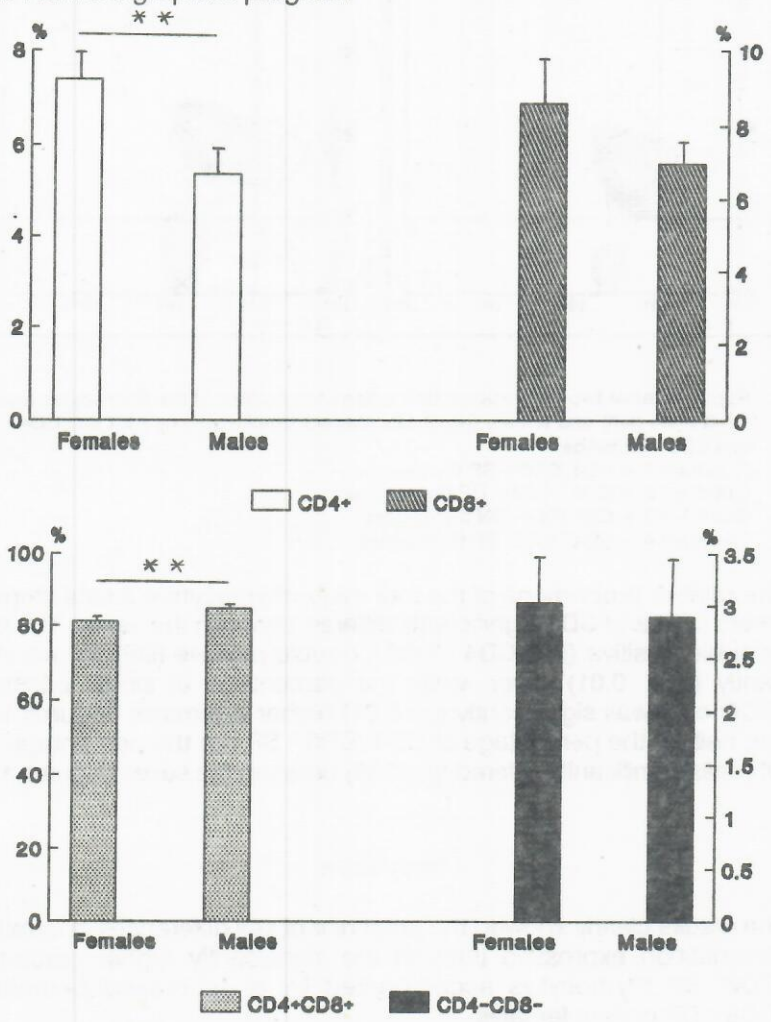

Figure 1. Percentage of $CD4^+/CD8^-$ SP, $CD4^-/CD8^+$ SP, $CD4^+/CD8^+$ DP and $CD4^-/CD8^-$ DN thymocytes isolated from adult female and male rats of the same age as determined by FCA.
n = 15
** $p < 0.01$

RESULTS

The yield of thymocytes extracted from female ($5.97 \times 10^8 \pm 0.92 \times 10^8$) and male rats ($7.46 \times 10^8 \pm 2.10 \times 10^8$) did not differ significantly ($p \geq 0.05$).

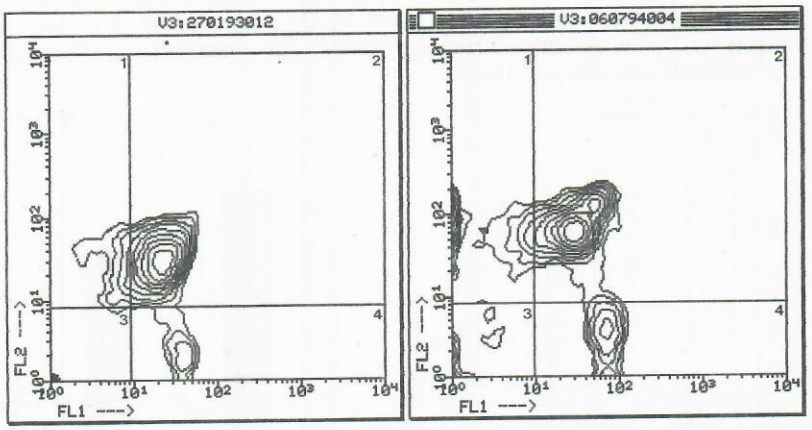

Figure 2. Representative two dimensional flow cytometric profiles of the thymocytes isolated from adult male (left) and female (right) rats stained simultaneously with anti CD4 - FITC and anti CD8 - PE mAbs.
Quadrant 1 = $CD4^-/CD8+$ SP thymocytes;
Quadrant 2 = $CD4^+/CD8+$ DP thymocytes;
Quadrant 3 = $CD4^-/CD8-$ DN thymocytes;
Quadrant 4 = $CD4^+/CD8-$ SP thymocytes

The relative proportions of the four major thymocyte subsets identified by the markers CD4 and CD8 significantly differed between the sexes. The percentage of double positive (DP) $CD4^+/CD8^+$ double positive (DP) thymocytes was significantly ($p \leq 0.01$) lower, while the percentage of single positive (CD) $CD4^+/CD8^-$ cells was significantly ($p \leq 0.01$) higher in females (Figures 1 and 2). However, neither the percentage of $CD4^-/CD8^+$ SP nor the percentage of $CD4^-/CD8^-$ DN cells significantly differed ($p \leq 0.05$) between the sexes (Figures 1 and 2).

DISCUSSION

The results clearly showed the presence of sex differences in intrathymic T cell differentiation expressed through the significantly higher percentage of $CD4^+/CD8^-$ SP thymocytes accompanied by a decreased percentage of $CD4^+/CD8^+$ DP cells in females.

These findings are consistent with data indicating that estrogens affect T cell differentiation, especially the helper T cell subset, increasing the percentage of $CD4^+/CD8^-$ SP thymocytes (Screpanti et al., 1989; Kawashima et al., 1990). It has also been found that 17-β estradiol treatment causes a decrease in the

percentage of $CD4^+/CD8^+$ DP cells and therefore it may be assumed that this treatment accelerates the physiological process of intrathymic maturation (Screpanti et al., 1989). The increased percentage of $CD4^+/CD8^-$ SP thymocytes in females is also consistent with the well-known predominance of helper T cells and the higher CD4/CD8 ratio in the peripheral blood of these animals (Mylgranam et al., 1985; Nagel et al., 1981). Coupled with the increased CD4/CD8 ratio is the finding that spleen cells from females generate more IgM and IgG in culture than spleen cells from male rats (Grossman et al., 1993). This may be attributed to the increase in CD4 (T helper) cells present in females which can act on B cells to promote antibody secretion.

On the other hand, it has been shown that in males changes in androgen status cause alterations in thymocyte phenotypic profile and T lymphocyte function. The removal of androgens shifted the T cell balance toward the CD4 helper subset and administration of androgens changed the balance toward CD8 suppressor/cytotoxic T cell predominance (Olsen et al., 1991; Aboudkhil et al., 1991).

The differences in the intrathymic maturational sequence between sexes can be related not only to direct action of these hormones on the developing cells (Kovacs and Olsen, 1987; Screpanti et al., 1989), but also to distinct effects of these hormones on the activity of reticulo-epithelial cells (Kawashima et al., 1991; Leposavic et Micic, 1992). Namely, Dardenne et al. (1986) reported that castration and adrenalectomy decreased secretion of thymulin and increased secretion of a thymulin inhibitory substance (TIS), whereas Grossman et al. (1982) reported that estrogen, but not dihydrotestosterone replacement in vivo depressed the release of thymic hormones from the thymic reticulo-epithelial cells and therefore suggested the presence of higher thymulin and lower TIS levels in females than in males. According to the same authors the hypothetical outcome of this fact might be increased T-helper activity resulting in enhanced B-cell production of immunoglobulins. The present findings strongly support this hypothesis.

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POLNE RAZLIKE U FENOTIPSKIM KARAKTERISTIKAMA TIMOCITA PACOVA

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SADRŽAJ

Cilj ovih istraživanja je bio da se ispita postojanje eventualnih razlika u procesu sazrevanja T ćelija u timusu koje bi mogle biti podloga seksualnom dimorfizmu imunog odgovora. U pacova oba pola ispitivana je procentualna zastupljenost četiri osnovne subpopulacije timocita, definisane ekspresijom CD4 i CD8 antigena, metodom direktne dvokolorne protočne citofluorimetrije (FCA). Određivanjem ukupnog broja timocita pokazano je da se on ne razlikuje značajno statistički između polova. Međutim, u ženki je nađeno značajno smanjenje procenta $CD4^+/CD8^+$ dvostruko pozitivnih (DP) timocita. Ovo smanjenje je bilo praćeno proporcionalnim povećanjem procenta $CD4^+/CD8^-$ jednostruko pozitivnih (SP) ćelija. Ovaj nalaz ukazuje da je u ženki ravnoteža u intratimusnom sazrevanju T ćelija pomerena u korist subpopulacije pomažućih (helper/inducer) T ćelija i implicira da je ovakav odnos između timocitnih subpopulacija u ženki, bar delimično, odgovoran za fenomen imunološkog seksualnog dimorfizma.